*Am J Hum Genet* 35:1126–1138, 1983

# The Human Serum Paraoxonase/Arylesterase Polymorphism

HARRY W. ECKERSON,[1] COLLETTE M. WYTE, AND BERT N. LA DU

SUMMARY

The heterozygous human serum paraoxonase phenotype can be clearly distinguished from both homozygous phenotypes on the basis of its distinctive ratio of paraoxonase to arylesterase activities. A trimodal distribution of the ratio values was found with 348 individual serum samples, measuring the ratio of paraoxonase activity (with 1 M NaCl in the assay) to arylesterase activity, using phenylacetate. The three modes corresponded to the three paraoxonase phenotypes, A, AB, and B (individual genotypes), and the expected Mendelian segregation of the trait was observed within families. The paraoxonase/arylesterase activity ratio showed codominant inheritance. We have defined the genetic locus determining the aromatic esterase (arylesterase) responsible for the polymorphic paraoxonase activity as esterase-A (ESA) and have designated the two common alleles at this locus by the symbols *ESA*A* and *ESA*B*. The frequency of the *ESA*A* allele was estimated to be .685, and that of the *ESA*B* allele, 0.315, in a sample population of unrelated Caucasians from the United States. We postulate that a single serum enzyme, with both paraoxonase and arylesterase activity, exists in two different isozymic forms with qualitatively different properties, and that paraoxon is a "discriminating" substrate (having a polymorphic distribution of activity) and phenylacetate is a "nondiscriminating" substrate for the two isozymes. Biochemical evidence for this interpretation includes the cosegregation of the degree of stimulation of paraoxonase activity by salt and paraoxonase/arylesterase activity ratio characteristics; the very high correlation between both the basal (non-salt stimulated) and salt-stimulated paraoxonase activities with arylesterase activity; and the finding that phenylacetate is an inhibitor for paraoxonase activities in both A and B types of enzyme.

Received February 17, 1983; revised April 18, 1983.

This work was supported by grant GM27028 from the U.S. Public Health Service and a grant from the Michigan Heart Association. A preliminary report of this work has been presented (ECKERSON HW, WYTE CM, LA DU BN: Pharmacogenetic studies on human serum paraoxonase: relative paraoxonase/arylesterase activities. *Fed Proc* 40:724, 1981).

[1] All authors: Department of Pharmacology, University of Michigan, Ann Arbor, MI 48109.

## INTRODUCTION

Human serum paraoxonase (E.C.3.1.1.2) catalyzes the hydrolysis of the organophosphate paraoxon to the nontoxic products, *p*-nitrophenol and diethylphosphoric acid [1–3]. Paraoxonase is an aromatic esterase [1] that requires calcium for activity; it is inhibited by chelating agents such as EDTA [2, 3], and by the organomercurial sulfhydryl reagent, *p*-hydroxymercuribenzoate (PMB). Unlike serum cholinesterase, it is not irreversibly inhibited by organophosphates [3, 4], and, indeed, some of the latter are substrates for the enzyme. In Caucasian populations, serum paraoxonase activity is bimodally distributed [3, 5–11] and the level of activity appears to be inherited as a simple dominant Mendelian trait determined by two alleles at one autosomal locus [6, 9–11]. The "high" and "low" alleles [7, 8], designated $N^H$ and $N^L$ by Playfer et al. [6], have since been called *ESA*A* and *ESA*B* [11] to emphasize the qualitatively different properties of the isozymes. The B isozyme is more highly stimulated by NaCl [11] and usually has greater activity than the A isozyme; the two isozymes also have different apparent $K_m$ values [12], calcium requirements [7], and pH optima [8].

Arylesterase [13] of human serum is also designated "aromatic" esterase [4, 14] (E.C.3.1.1.2). Arylesterase activity has most often been measured with phenylacetate as the substrate [14–21], but many other aromatic esters are also hydrolyzed: *o*- and *p*-nitrophenyl acetate [14, 22], *beta*-naphthylacetate [13, 23], vinylacetate [24, 25], and thiophenylacetate [26]. Arylesterase activity is greatest for aromatic substrates with an acetate moiety [24]. Arylesterase, like paraoxonase, requires calcium for activity [20], is inhibited by PMB and EDTA [3, 27, 28], and is not inhibited by cholinesterase inhibitors such as eserine and organophosphates [29].

In spite of these similarities, there has been some doubt whether human serum paraoxonase and arylesterase activities are properties of the same enzyme or are two distinct enzymes. For example, an individual's level of arylesterase is under genetic control but not by a single major gene locus [18], and the distribution of arylesterase in a Caucasian population has a single mode [18], whereas paraoxonase activity is bimodal. Kinetic evidence has been cited to suggest that hydrolysis of aromatic esters and certain organophosphates is carried out by a single enzyme in serum [1]; however, with purification of sheep serum paraoxonase, the ability to hydrolyze phenylacetate was lost [30].

Our study shows that within a Caucasian population from the United States the ratio of serum paraoxonase activity to arylesterase activity was distributed trimodally. All three paraoxonase phenotypes (the individual genotypes) could be clearly identified by the ratio of paraoxonase/arylesterase activities. The paraoxonase/arylesterase ratio characteristic was inherited as a simple, autosomal Mendelian trait, without dominance, whereas, previously, the paraoxonase activity polymorphism was bimodally distributed. Furthermore, phenylacetate was an inhibitor of both the A- and B-type paraoxonase activity. These results are consistent with the hypothesis that a single gene locus determines the arylesterase/paraoxonase phenotypes of human serum, and, most likely, different isozymic forms of the enzyme accounts for the paraoxonase polymorphism and the different ratio of paraoxonase/arylesterase activities with the two substrates.

## MATERIALS AND METHODS

### *Serum Samples*

Serum or heparinized plasma samples were collected by venipuncture from 348 normal healthy donors from the Ann Arbor, Michigan, area. The samples were stored frozen at −20°C until assay, usually within 2 weeks. The population included: 181 males, 167 females, 324 Caucasians, 23 Asians, and one black American.

### *Partially Purified Paraoxonase/Arylesterase*

For some experiments, a preparation of enzyme was purified 60–80-fold over serum. Each ml of serum was precipitated with 182 U of heparin and 92 nmol $MnCl_2$ for 30 min and the supernatant collected after centrifugation (30 min; 4°C; 500 *g*) [31]. The heparin-manganese supernatant was applied to a reactive blue agarose (Sigma, St. Louis, Mo.) column [32] in 1 M NaCl in column buffer (pH 8.0, 50 mM Tris/HCl containing 1 mM $CaCl_2$ and 0.005 mM EDTA [21]). The column was washed with 4 M NaCl in column buffer and then eluted with 0.2% sodium deoxycholate dissolved in water. The eluted fraction had 50%–70% of the initial paraoxonase and arylesterase activities.

### *Arylesterase Activity*

Arylesterase activity was measured using phenylacetate as the substrate by the procedure of Zeller [33], as modified by Kitchen et al. [21]. Initial rates of hydrolysis were determined spectrophotometrically at 270 nm. Added to 3.0 ml of a 25°C reaction mixture was 0.005 ml of sample for a 1:601-fold dilution. The reaction mixture contained 1 mM phenylacetate (Sigma), 9 mM, pH 8.0, Tris/HCl, and 0.9 mM $CaCl_2$. The nonenzymatic hydrolysis of phenylacetate was subtracted from the total rate of hydrolysis. The $E_{270}^{M}$ for the reaction was 1,310. One U of arylesterase activity equaled 1 mmol of phenylacetate hydrolyzed per min. Usually, the concentration of arylesterase activity was expressed as units per ml of serum.

The effect of NaCl upon arylesterase activity was determined using the above reaction mixture containing NaCl to give final concentrations from 0–1.0 M.

### *Paraoxonase Activity Assay*

Paraoxonase assays were made either without any added NaCl (basal activity) or with 1 M NaCl included (salt-stimulated activity) [11]. The rate of hydrolysis of paraoxon was assessed by measuring the liberation of *p*-nitrophenol at 412 nm at 25°C. The basal assay mixture included 1.0 mM paraoxon and 1.0 mM $CaCl_2$ in 0.05 M glycine buffer, pH 10.5, and no additional NaCl. One U of paraoxonase activity produced 1 nmol of *p*-nitrophenol per min, and activity was usually expressed as units per ml serum.

For studying the kinetics of interaction between paraoxon and phenylacetate, paraoxonase activities were measured at pH 8.0, with 10 mM Tris/HCl buffer containing 1 mM $CaCl_2$. Paraoxon concentrations ranged from 0.1 mM to 2.0 mM. The $E_{412}^{M}$ was 16,900. Initial rates were linear for at least 5 min at the lowest concentration of substrate, paraoxon, and the lowest concentration of inhibitor, phenylacetate.

### *Percent Stimulation of Paraoxonase Activity*

The percent stimulation of paraoxonase by 1 M NaCl (percent stimulation characteristic) was expressed as:

$$\frac{\text{Paraoxonase activity with 1 M NaCl} - \text{basal paraoxonase activity}}{\text{Basal paraoxonase activity}} \times 100\% .$$

Individuals were classified for paraoxonase phenotype [11] using the antimode at 60% stimulation as the dividing point between the non-salt-stimulated, A type, and the salt-stimulated, AB and B types.

*Paraoxonase to Arylesterase Activity Ratio*

We defined an individual's paraoxonase/arylesterase ratio characteristic as the ratio of salt-stimulated paraoxonase activity at pH 10.5 to arylesterase activity with phenylacetate as substrate.

$$\text{Ratio} = \frac{\text{Paraoxonase activity with 1 M NaCl}}{\text{Arylesterase activity}}$$

## RESULTS

*Population Distribution of Paraoxonase, Arylesterase, and Their Ratio*

The distribution of individuals with respect to arylesterase activity was unimodal (fig. 1*A*). The paraoxonase activity of these individuals was bimodally distributed (fig. 1*B*), and the distribution of the ratio of paraoxonase to arylesterase activities was clearly trimodal (fig. 1*C*). A tentative assignment of individuals within three possible paraoxonase phenotypes was made based on the ratio of paraoxonase to arylesterase activities, dividing the populations at the antimodes (1.8 and 6.9; fig. 1*C*). The paraoxonase activity used for all of the above was measured with 1 M NaCl in the assay. If basal paraoxonase activity, without added salt, were used, the two higher modes of ratio would be less, whereas the A type, lowest mode, would not change. As a consequence, the discrimination between the upper modes would be decreased (no figure presented).

*Estimation of Gene Frequency and Test Whether the Three Ratio Types Fit a Hardy-Weinberg Equilibrium Distribution*

If the three modes of the paraoxonase/arylesterase ratio correspond to the three paraoxonase phenotypes (individual genotypes), there are 101 unrelated Caucasian individuals of the A phenotype, 92 of the heterozygous, AB phenotype, and 22 of the B phenotype. From the frequency of the A phenotype, the gene frequencies can be estimated as .685 and .315 for the *ESA*A* and *ESA*B* alleles, respectively.

Using these gene frequencies and assuming the population to be in Hardy-Weinberg equilibrium, the number of individuals who should be of the AB and B phenotypes was estimated. The predicted number of AB and B types was 92.72 and 21.28, in excellent agreement with the observed numbers ($\chi^2 = 0.03$; $P > .8$).

*The Ratio of Paraoxonase to Arylesterase Activity*

In figure 2, the individual paraoxonase and arylesterase activities are presented from which the paraoxonase/arylesterase ratio characteristic was calculated. The paraoxonase/arylesterase ratio characteristic and the level of salt-stimulated paraoxonase activity are not randomly distributed but are interdependent, as expected for one trait. The arylesterase activity of A phenotype individuals, who can be well distinguished by salt-stimulation alone, correlated very highly ($r = .856$; $P < .001$; no. = 156) with paraoxonase activity (fig. 2, symbol = ▲). This population of individuals was clearly separated from the salt-stimulated B type and represented a single paraoxonase genotype, the homozygous *ESA*A* type [11]. Using salt-stimulated activity alone, the salt-stimulated individuals

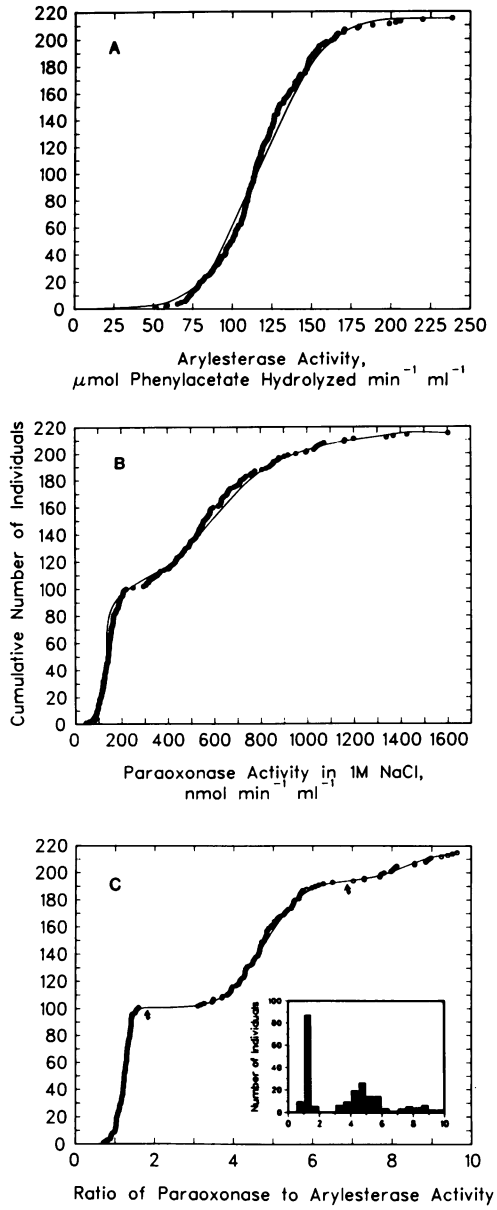


FIG. 1.—The population distribution of paraoxonase and arylesterase activities and their ratio. The cumulative distributions of 215 unrelated Caucasians with respect to their arylesterase activity (*A*), paraoxonase activity with 1 M NaCl (*B*), and the ratio of these two activities (*C*) are presented. *Solid line* is theoretical distribution for a randomly selected population that is in Hardy-Weinberg equilibrium at the ESA locus for the *A* and *B* alleles and in which the alleles are in a ratio of .685:.315. The value for the population of AB phenotype is assumed to be an additive function of the *ESA*A* and *ESA*B* products. The population separated into three distinct groups only in figure 1*C*. *Arrows* at ratios of 1.8 and 6.9 are where we divide the phenotypes. *Insert* in figure 1*C* is a histogram of the same individuals as presented in the cumulative distribution. Similar patterns were obtained using all 348 individuals, including both related and unrelated individuals of all races (not illustrated).

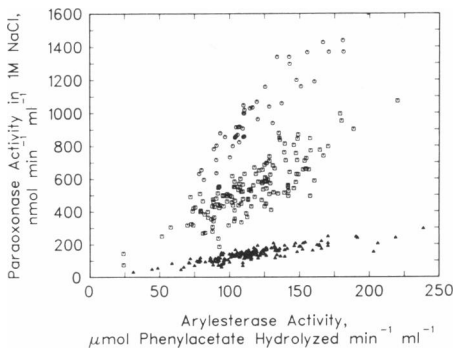


FIG. 2.—Individual paraoxonase and arylesterase activities. A plot of individual paraoxonase activity with 1 M NaCl vs. arylesterase activity shows three groups of individuals: ▲, □, ○; these correspond to the three paraoxonase phenotypes, A, AB, and B, respectively. All of the 348 individuals tested are presented.

included both homozygous, B, and heterozygous, AB, phenotypes. The salt-stimulated individuals appeared to consist of two distinct populations (fig. 2, symbols ○ and □). Within each of the two salt-stimulated populations, there was a very high correlation of arylesterase and paraoxonase activities (r = .938 and .829; $P$ = .001; no. = 45 and 144, within each group, designated ○ and □, fig. 2). Even without added salt to the paraoxonase activity assay, there was a very high correlation of paraoxonase activity and arylesterase activity within the three groups (r = .822, .822, and .825, for the A, AB, and B phenotypes, respectively; $P < .001$).

### *Pedigree Studies to Determine whether the Paraoxonase/Arylesterase Ratio Characteristic Is Inherited as a Simple Mendelian Trait*

Pedigree studies (table 1) showed that the paraoxonase/arylesterase ratio characteristic followed the expected pattern for Mendelian inheritance of two alleles at one autosomal locus. A sex-linked model was excluded because in our unrelated Caucasian population the numbers of males and females of each phenotype were: A type, 59 and 42; B type, 14 and 8; AB type, 45 and 47, respectively. Among the very highly informative mating types, no progeny were misclassified. In addition, the frequency of the different mating types agreed closely with the expected values (excluding two Oriental families) if the population follows a Hardy-Weinberg distribution and the gene frequencies are .685 and .315, as given above. The distribution of parental mating types observed were not significantly different from the theoretical values (G = 1.38; calculations included the Yates' correction because the number of families here was only 36; df = 5; $P > .9$).

Two of the families studied were Chinese. The mother in one was of A type, and the father, AB. Of the progeny from this family, one boy and one girl were of AB type, another girl was A type. In the other family, the father's phenotype was AB and the mother's was B. There were three B-type progeny, two girls and one boy, and one AB-type girl. Although these pedigrees are small, the data suggest that the ESA polymorphism also occurs in the Oriental population, and

TABLE 1

PEDIGREE ANALYSIS OF THE PARAOXONASE/ARYLESTERASE RATIO CHARACTERISTIC SHOWS CODOMINANT INHERITANCE OF TWO ALLELES AT ONE LOCUS

| MATING TYPES | NO. FAMILIES | NO. PROGENY OF EACH PHENOTYPE | | | | | | LIKELIHOOD RATIO FOR PROGENY |
|---|---|---|---|---|---|---|---|---|
| | | A | | AB | | B | | |
| | | Expected | Observed | Expected | Observed | Expected | Observed | |
| A × A | 6 | 14 | 14 | 0 | 0 | 0 | 0 | |
| AB × B | 7 | 0 | 0 | 7.5 | 5 | 7.5 | 10 | |
| Subtotal | | 14 | 14 | 7.5 | 5 | 7.5 | 10 | 2.85 |
| B × B | 1 | 0 | 0 | 0 | 0 | 3 | 3 | |
| A × AB | 17 | 17 | 19 | 17 | 15 | 0 | 0 | |
| Subtotal | | 17 | 19 | 17 | 15 | 3 | 3 | 1.96 |
| AB × AB | 4 | 2 | 3 | 4 | 5 | 2 | 0 | |
| A × B | 3 | 0 | 0 | 6 | 6 | 0 | 0 | |
| Subtotal | | 2 | 3 | 10 | 11 | 2 | 0 | 1.46 |
| Grand total | 38 | | 36 | | 31 | | 13 | $G = 4.12$ ($df = 5, P > 0.2$) |

NOTE: Maximum likelihood calculated using Yates' correction.

individual phenotypes can be assigned using the paraoxonase/arylesterase ratio characteristic. This had not been apparent from the earlier work of Playfer et al. [6], who found a unimodal distribution of paraoxonase activity in an Oriental population.

The phenotype assignment using either the paraoxonase/arylesterase ratio or percent stimulation of paraoxonase activity by 1 M NaCl gave virtually identical results: 159 vs. 161 individuals of the A type and 189 vs. 187 of the AB plus B types (the population included all individuals, related and unrelated). One of the two individuals misclassified using percent salt stimulation had low paraoxonase activity, so that the contribution of albumin to the total rate of hydrolysis may have been given greater weight than usual. The average rate of paraoxon hydrolysis in the presence of 1 mM EDTA (without added NaCl or $CaCl_2$) is 1.73 ± 0.97 and 1.50 ± 0.86 nmol $min^{-1}$ $ml^{-1}$, respectively, for six individuals of the A and six of the B phenotype ($P$ about .70; not significant; less than 2% of the rate with calcium in an average individual). The paraoxonase/arylesterase ratio and salt-stimulation characteristics cosegregated within pedigrees, as would be expected if they are measures of the same trait. The paraoxonase/arylesterase ratio characteristic allows the unequivocal assignment of all three phenotypes, whereas measurement of paraoxonase activity alone [6, 8–10], or percent salt stimulation [11], did not clearly discriminate between AB and B phenotypes.

*Effect of NaCl on Arylesterase Activity*

Figure 3 shows that arylesterase activity was decreased by concentrations of NaCl that stimulated the paraoxonase activity of the B type. There was a small but significant difference between the response of the two phenotypes. The arylesterase activity of the two phenotypes appeared to respond in a qualitatively different way to NaCl, but the difference was much smaller than with paraoxonase activity.

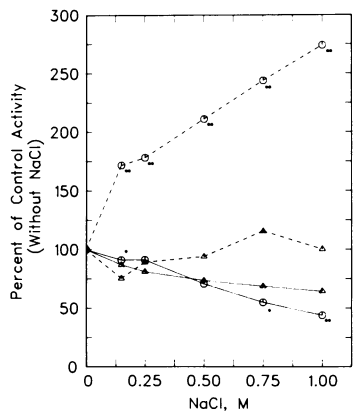


FIG. 3.—Inhibition of arylesterase activity by NaCl. Six samples of A type (△) and six of B type (○) were tested for their response to NaCl using phenylacetate as the substrate, *solid lines*. *Dashed lines* were those obtained using paraoxon as the substrate (an average of the individuals in figure 2 of Eckerson et al. [11]). The response of the A and B types differed at the $P < .05$ level (*) and $P < .01$ level (**) as indicated. NaCl has been observed to increase the activity of the B-phenotype paraoxonase activity [11], but inhibit arylesterase activity with both phenotypes.

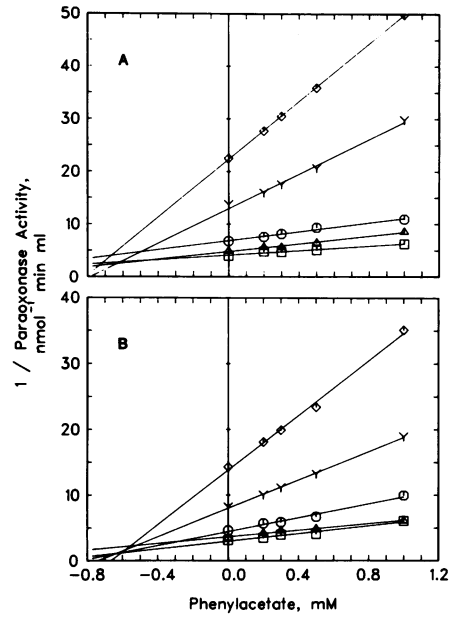


FIG. 4.—The inhibition of paraoxon hydrolysis by phenylacetate. Phenylacetate is an inhibitor of paraoxonase activity using either the A or B type partially purified paraoxonase/arylesterase activity. The concentrations of paraoxon used were 2.0 (□), 1.0 (△), 0.5 (○), 0.2 (Y), and 0.1 (◇) mM, and the concentrations of phenylacetate were 1.0, 0.5, 0.3, 0.2, and 0 mM. The assays were carried out at pH 8.0. From the slope of the Dixon plot of 1/paraoxonase activity vs. phenylacetate concentration, the Ki for the A phenotype was determined to be 0.77 mM and 0.55 mM for the B phenotype.

### *Interaction of Paraoxon and Phenylacetate*

Our hypothesis that both paraoxon and phenylacetate are hydrolyzed by the same enzyme was supported by kinetic studies of paraoxon hydrolysis in which phenylacetate was used as an inhibitor. Figure 4 shows that phenylacetate behaved as an inhibitor of paraoxonase activity with both the A- and B-type enzymes. The type of inhibition was predominantly competitive as the extrapolated lines on the Dixon plot intersected, but the plot of the Dixon slope vs. phenylacetate concentration passed slightly above zero, and so the inhibition may not be purely competitive.

### *Characterization of the Paraoxonase Phenotypes*

Table 2 shows both the activities, percent stimulation, and activity ratio for sera representing the three types of paraoxonase (where phenotype was assigned using each individual's paraoxonase/arylesterase ratio). For the AB phenotype, a theoretical value is also given for each of these parameters, assuming a simple, additive model, without interaction of the gene products. None of the observed values differs significantly from the additive model (using analysis of variance or Student's *t*-test).

## DISCUSSION

Although the paraoxonase polymorphism has been recognized for several years [5–11], no human serum arylesterase polymorphism has been previously reported.

Simpson observed that the level of serum arylesterase activity is primarily determined by genetic factors [18], and both we (fig. 1A) and Simpson [18] found arylesterase activity to be distributed as a single mode. The striking correlation between arylesterase activity (measured using phenylacetate as the substrate) and paraoxonase activity within the three phenotypes suggested that the two activities might well be properties of the same enzyme. The trimodal distribution for the ratio of paraoxonase to arylesterase activity (fig. 1*C*) and the inheritance of this trait as a simple Mendelian characteristic at the ESA locus (table 1) indicate that paraoxonase and arylesterase are more than casually related enzyme activities.

The ratio of paraoxonase to arylesterase activity gives a clear separation of all three paraoxonase phenotypes with no overlap of the groups; all three genotypes can be assigned. The measurement of paraoxonase activity alone either directly [5, 6, 8] or indirectly [9, 10], even with 1 M NaCl in the assay [11], or varying calcium concentration [7], distinguished only two phenotypes. Previously, a heterozygous AB-type individual was distinguished from a homozygous B phenotype by statistical methods [10, 34]. However, the paraoxonase/arylesterase ratio characteristic makes the statistical division of the upper paraoxonase mode no longer necessary. In retrospect, the assignment of the B phenotype using paraoxonase activity was probably usually correct because the mean for the homozygote B type is greater than that of the heterozygote (table 2), both with 1 M NaCl in the assay and without it. The gene frequencies for our Caucasian population are similar to those using only the bimodally distributed activity measurements. The gene frequency for the *ESA*A* allele in our population from the

TABLE 2

CHARACTERISTICS OF THE PARAOXONASE/ARYLESTERASE PHENOTYPES

| | PARAOXONASE/ARYLESTERASE PHENOTYPE | | | |
|---|---|---|---|---|
| | | AB | | |
| | A | Observed | Expected | B |
| No. | 159 | 144 | ... | 45 |
| Age, yrs | 33 ± 15 | 32 ± 16 | ... | 33 ± 14 |
| Sex, % males | 55% | 47% | ... | 60% |
| Paraoxonase activity (nmol $min^{-1}$ $ml^{-1}$): | | | | |
| –NaCl | 135 ± 43 | 228 ± 61 | 238 ± 64 | 341 ± 79 |
| +1 M NaCl | 140 ± 43 | 539 ± 170 | 574 ± 175 | 1,008 ± 244 |
| Arylesterase activity (μmol $min^{-1}$ $ml^{-1}$) | 116 ± 33 | 115 ± 30 | 119 ± 31 | 121 ± 29 |
| % stimulation of paraoxonase | 5.1 ± 11 | 135 ± 32 | 141 ± 24 | 194 ± 32 |
| Paraoxonase/arylesterase ratio | 1.21 ± 0.19 | 4.68 ± 0.85 | 4.84 ± 0.51 | 8.36 ± 0.70 |

NOTE: Phenotype was assigned using the paraoxonase/arylesterase ratio. The expected results for the AB phenotype are those for an additive model with equal contribution from each allele product and no interaction of the alleles or products.

United States was .685; others have reported frequencies of: .7034, for an English population [6]; .7595 for a German population [34]; .73 for a Canadian population (estimated from the data of Carro-Ciampi et al. [7]); and .726 for a Danish population [8]. The paraoxonase/arylesterase ratio characteristic should also be useful in estimating the frequency of the *ESA*A* and *ESA*B* alleles in different ethnic populations in which the frequency of the ESA alleles varies from the Caucasian or in which other variants may occur. Playfer et al. [6] showed the paraoxonase activity of Oriental and black African populations to be distributed quite differently from that of the Caucasian population. From the limited data of our two Chinese pedigrees, there was no apparent difficulty in using the ratio characteristic to classify individuals. Perhaps the *ESA*A* and *ESA*B* alleles are segregating in the Chinese as they do in the Caucasian population.

The inhibition of paraoxon hydrolysis by phenylacetate was not unexpected, if phenylacetate and paraoxon act at a common site in the active center of the enzyme. A single active site hydrolyzing both substrates would also explain the high degree of correlation between the arylesterase and paraoxonase activities within each phenotype. Paraoxon appeared to be a "discriminating" substrate for the A and B isozymes under most conditions of assay, whereas phenylacetate was "nondiscriminating" (hydrolyzed at similar rates by the isozymes). Possibly this finding is analogous to the situation with human liver *N*-acetyltransferase: *p*-aminobenzoic acid is a nondiscriminating (monomorphic) substrate, and isoniazid, a discriminating (polymorphic) substrate [35, 36]. Apparently, the turnover of the A and B isozymes differ with paraoxon but are similar with phenylacetate. One possible model that would explain this difference is that the rate-limiting step in paraoxon hydrolysis is more rapid with the B isozyme; but for phenylacetate, this is not the case. In fact, the rate-limiting step for phenylacetate hydrolysis might be different from that for paraoxon. Since the rate of hydrolysis of phenylacetate is so much greater (about 1,000-fold) than that of paraoxon, different rate-limiting steps would not be surprising. Both genetic and biochemical evidence led us to postulate that phenylacetate (and probably several other aromatic substrates) are hydrolyzed by the same human serum enzyme as the one that hydrolyzes paraoxon.

Various lines of evidence support the conclusion that the ESA locus determines one serum enzyme having two common isozymic forms. The ratio of paraoxonase to arylesterase activities clearly divides a population from the United States into three modes that correspond to the three paraoxonase phenotypes, A, AB, and B; the characteristic is inherited as a simple Mendelian trait at the ESA locus. The *ESA*A* and *ESA*B* alleles appear to determine qualitatively different serum paraoxonase/arylesterase isozymes that respond differently to salt [11] and calcium [7], and which have different apparent $K_m$ [12] and pH optima [8]. Preliminary experiments in our laboratory have also shown that chlorpromazine can differentially inhibit arylesterase, distinguishing the phenotypes [37].

While the substrate specificity of purified human serum paraoxonase/arylesterase has not been established, that of animals has been studied. Bovine serum arylesterase has been purified by a number of groups [15–17, 21], but no one has reported whether paraoxon was hydrolyzed by their purified preparation. The one species

from which paraoxonase activity has been highly purified is sheep serum [38]. Main found a purified preparation of sheep serum paraoxonase hydrolyzed *p*-nitrophenylacetate and related compounds but not phenylacetate [30]. It is possible that these characteristics are due to an unusual type of species difference. As is well known, esterases of different species differ markedly from each other, and from those of man [27, 29]. Some animal species have many more serum esterase activities and esterase types, such as aliesterases, of which man has none [25]. Proof of the one enzyme hypothesis will require purification of these activities from human serum, and this is in progress in our laboratory [39].

## REFERENCES


1. ALDRIDGE WN: Serum esterases 2. An enzyme hydrolyzing diethyl *p*-nitrophenyl phosphate (E600) and its identity with the A-esterase of mammalian sera. *Biochem J* 53:117–124, 1953
2. ERDÖS EG, BOGGS LE: Hydrolysis of paraoxon in mammalian blood. *Nature* 190:716–717, 1961
3. KRISCH K: Enzymatische Hydrolyse von Diathyl-p-nitrophenylphosphat (E600) durch menchliches Serum. *Z Klin Chem Klin Biochem* 6:41–45, 1968
4. ALDRIDGE WN, REINER E: A-esterases, in *Enzyme Inhibitors as Substrates: Interactions of Esterases with Esters of Organophosphorous and Carbamic Acids*, edited by NEUBERGER A, TATUM EL, Frontiers of Biology Series, New York, American Elsevier, 1975, pp 176–189
5. ZECH R, ZÜRCHER K: Organophosphate splitting serum enzymes in different mammals. *Comp Biochem Physiol [B]* 48:427–433, 1974
6. PLAYFER JR, EZE LC, BULLEN MF, EVANS DAP: Genetic polymorphism and interethnic variability of plasma paraoxonase activity. *J Med Genet* 13:337–342, 1976
7. CARRO-CIAMPI G, KADAR D, KALOW W: Distribution of serum paraoxon hydrolyzing activities in a Canadian population. *Can J Physiol Pharmacol* 59:904–907, 1981
8. EIBERG H, MOHR J: Genetics of paraoxonase. *Ann Hum Genet* 45:323–330, 1981
9. GELDMACHER-V. MALLINCKRODT M, LINDORF HH, PETENYI M, FLUGEL M, FISCHER T, HILLER T: Genetisch determinierter Polymorphismus der menschlichen Serum-Paraoxonase (EC 3.1.1.2). *Humangenetik* 17:331–335, 1973
10. GELDMACHER-V. MALLINCKRODT M, HOMMEL G, DUMBACH J: On the genetics of the human serum paraoxonase (EC 3.1.1.2). *Hum Genet* 50:313–326, 1979
11. ECKERSON HW, ROMSON J, WYTE C, LA DU BN: The human serum paraoxonase polymorphism. Identification of phenotypes by their response to salts. *Am J Hum Genet* 35:214–227, 1983
12. FLÜGEL M, GELDMACHER-V. MALLINCKRODT M: Zur Kinetik des Paraoxon-spaltenden Enzyms im menschlichen Serum (EC 3.1.1.2). *Klin Wochenschr* 56:911–916, 1978
13. AUGUSTINSSON KB: Electrophoretic separation and classification of blood plasma esterases. *Nature* 181:1786–1789, 1958
14. MOUNTER LA, WHITTAKER VP: The hydrolysis of esters of phenol by cholinesterases and other esterases. *Biochem J* 54:551–559, 1953
15. DON MM, MASTERS CJ, WINZOR DJ: Further evidence for the concept of bovine plasma arylesterase as a lipoprotein. *Biochem J* 151:625–630, 1975
16. CHOI SS, FORSTER TL: Triton X-155 as a stabilizer of bovine plasma arylesterase activity. *J Dairy Sci* 50:837–839, 1967
17. CHOI SS, FORSTER TL: Purification of bovine plasma arylesterase. *J Dairy Sci* 50:1088–1091, 1967
18. SIMPSON NE: Serum arylesterase levels of activity in twins and their parents. *Am J Hum Genet* 23:375–382, 1971
19. MARTON AV, KALOW W: Interaction between aromatic esterase of human serum and bivalent metal ions. *Can J Biochem Physiol* 40:319–324, 1962

20. ERDÖS EG, DEBAY CR, WESTERMAN MP: Arylesterases in blood: effect of calcium and inhibitors. *Biochem Pharmacol* 5:173–186, 1960
21. KITCHEN BJ, MASTERS CJ, WINZOR DJ: Effects of lipid removal on the molecular size and kinetic properties of bovine plasma arylesterase. *Biochem J* 135:93–99, 1973
22. HUGGINS C, LAPIDES J: Chromogenic substrates. IV. Acyl esters of *p*-nitrophenol as substrates for the colorimetric determination of esterase. *J Biol Chem* 170:467–482, 1947
23. BURLINA A, MICHIELIN E, GALZIGNA L: Characteristics and behaviour of arylesterase in human serum and liver. *Eur J Clin Invest* 7:17–20, 1977
24. AUGUSTINSSON KB, EKEDAHL G: On the specificity of arylesterases. *Acta Chem Scand* 16:240–241, 1962
25. AUGUSTINSSON KB: Arylesterases. *J Histochem Cytochem* 12:744–747, 1964
26. MENDOZA CE, SHIELDS JB, AUGUSTINSSON KB: Arylesterases from various mammalian sera in relation to cholinesterases, carboxylesterases and their activity towards some pesticides. *Comp Biochem Physiol [C]* 55:23–26, 1976
27. AUGUSTINSSON KB: Multiple forms of esterase in vertebrate blood plasma. *Ann NY Acad Sci* 94:844–860, 1961
28. BLANKO A, ZINKHAM WH: Soluble esterases in human tissues: characterization and ontogeny. *Bull Johns Hopkins Hosp* 118:27–39, 1966
29. LA DU BN, SNADY H: *Esterases of Human Tissues*, in *Handbook of Experimental Pharmacology New Series*, vol XXVIII/2, edited by BRODIE BB, GILLETTE J, Berlin, Springer-Verlag, 1971, pp 477–499
30. MAIN AR: The differentiation of the A-type esterases in sheep serum. *Biochem J* 75:188–195, 1960
31. BACHORIK PS, WOOD PD, ALBERS JJ, ET AL.: Plasma high-density lipoprotein cholesterol concentrations determined after removal of other lipoproteins by heparin/manganese chloride precipitation or by ultracentrifugation. *Clin Chem* 22:1828–1834, 1976
32. WILLE LE: A simple and rapid method for isolation of serum lipoproteins from other serum protein constituents. *Clin Chim Acta* 71:355–357, 1976
33. ZELLER EA: Enzymology of the refractory media of the eye. IV. Direct photometric determination of cholinesterase and aliesterase of corpus vitreum. *Arch Biochem Biophys* 61:231–240, 1956
34. GELDMACHER-V. MALLINCKRODT M: Polymorphism of human serum paraoxonase. *Hum Genet* (Suppl 1):65–68, 1978
35. PATTERSON E, RADTKE HE, WEBER WW: Immunochemical studies of rabbit N-acetyltransferases. *Mol Pharmacol* 17:367–373, 1980
36. HEIN DW, HIRATA M, GLOWINSKI IB, WEBER WW: Biochemical evidence for the coexistence of monomorphic and polymorphic N-acetyltransferase activities on a common protein in rabbit liver. *J Pharmacol Exp Ther* 220:1–7, 1982
37. ECKERSON HW, STERN S, LA DU BN: Chlorpromazine: a differential inhibitor of human serum arylesterases A and B. *Am J Hum Genet* 34:50A, 1982
38. MAIN AR: The purification of the enzyme hydrolyzing diethyl-*p*-nitrophenyl phosphate (paraoxon) in sheep serum. *Biochem J* 74:10–20, 1960
39. SMOLEN A, ECKERSON HW, LA DU BN: Purification of arylesterases A and B from human serum. *Am J Hum Genet* 34:63A, 1982